IN THE SPECIFICATION

Please insert the following replacement paragraph starting at page 2, line 9:

B1

In one aspect, the present invention provides recombinant nucleic acid molecules that encode a fusion polypeptide, the recombinant nucleic acid molecules comprising a Ra12 polynucleotide sequence and a heterologous polynucleotide sequence, wherein the Ra12 polynucleotide sequence hybridizes to SEQ ID NO:3 under stringent conditions. In one embodiment, the recombinant nucleic acid molecules comprise a Ra12 polynucleotide sequence which is located 5' to a heterologous polynucleotide sequence. In another embodiment, the recombinant nucleic acid molecules further comprise a polynucleotide sequence that encodes a linker peptide between the Ra12 polynucleotide sequence and the heterologous polynucleotide sequence, wherein the linker peptide may comprise a cleavage site. In yet another embodiment, the recombinant nucleic acid molecules encode fusion polypeptides which further comprise an affinity tag. In yet another embodiment, the recombinant nucleic acid molecules encode a fusion polypeptide comprising a D Purified Protein Derivative ("DPPD"), a "Wilm's Tumor Gene ("WT1"), a mammaglobin, or a H9-32A heterologous polypeptide. In yet another embodiment, the recombinant nucleic acid molecules comprise a Ra12 polynucleotide sequence comprising at least about 30 nucleotides, at least about 60 nucleotides, or at least about 100 nucleotides. In yet another embodiment, the recombinant nucleic acid molecules comprise a Ra12 polynucleotide sequence as shown in SEQ ID NO:3. In yet another embodiment, the recombinant nucleic acid molecules comprise a Ra12 polynucleotide sequence that encodes a Ra12 polynucleotide as shown in SEQ ID NO:4, SEQ ID NO:17 or SEQ ID NO:18.

Please insert the following replacement paragraph starting at page 4, line 29:

B2

Surprisingly, it was discovered by the present inventors that a 14 KD C-terminal fragment of the MTB32A coding sequence expresses at high levels on its own and remains as a soluble protein throughout the purification process. This 14 KD C-terminal fragment of the MTB32A is referred herein as Ra12 (having amino acid residues

B2 Conclude

192 to 323 of MTB32A). The nucleic acid and amino acid sequences of native Ra12 are shown, *e.g.*, in Figures 2-6. As described in detail below, the term "Ra12 polypeptide" or "Ra12 polynucleotide" as used herein refer to the native Ra12 sequences (*e.g.*, SEQ ID NO:4 or SEQ ID NO:3, respectively), their variants, or fragments thereof (e.g., SEQ ID NO:17 or SEQ ID NO:18). The present invention utilizes these properties of Ra12 polypeptides and provides recombinant nucleic acid molecules, expression vectors, host cells, and methods for stable and high yield expression of fusion polypeptides comprising a Ra12 polypeptide and a heterologous polypeptide of interest. The materials and methods of the present invention are particularly useful in expressing certain heterologous polypeptides (*e.g.*, DPPD) that other conventional expression methods failed to express in any substantial quantity.

Please insert the following replacement paragraph starting at page 6, line 22:

B3

Polynucleotides may comprise a native sequence (*i.e.*, an endogenous sequence that encodes a Ra12 polypeptide SEQ ID NO:3 or a portion thereof) or may comprise a variant of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the biological activity of the encoded fusion polypeptide is not diminished, relative to a fusion polypeptide comprising a native Ra12 polypeptide. Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native Ra12 polypeptide (SEQ ID NO:4) or a portion thereof. Optionally, the identity exists over a region that is at least about 25 to about 50 nucleotides in length, at least about 75-100 nucleotides in length, or a nucleotide sequence encoding at least about 25 to about 50 amino acids, or a nucleotide sequence encoding at least about 75- 100 amino acids.

Please insert the following replacement paragraph starting at page 8, line 29:

B4

The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of

B4 Conclude

nucleic acid, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes*, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10 °C lower than the thermal melting point ($T_m$) for the specific sequence at a defined ionic strength and pH. The $T_m$ is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at $T_m$, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, preferably 10 times background hybridization. Exemplary stringent hybridization conditions can be as following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with a wash in 0.2x SSC, and 0.1% SDS at 65°C.

Please insert the following replacement paragraph starting at page 12, line 23:

B5

Depending on the host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used in the expression vector. For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage λ, plac, ptrp, ptac (ptrp-lac hybrid promoter; cytomegalovirus promoter) and the like may be used; when cloning in yeast cell systems, promoters such as ADHI, PGK, PHO5, or the α factor promoter may be

used; when cloning in insect cell systems, promoters such as the baculovirus polyhedron promoter may be used; when cloning in plant cell systems, promoters derived from the genome of plant cells (*e.g.*, heat shock promoters; the promoter for the small subunit of RUBISCO; the promoter for the chlorophyll $\alpha/\beta$ binding protein) or from plant viruses (*e.g.*, the 35S RNA promoter of cauliflower mosaic virus ("CaMV"); the coat protein promoter of tobacco mosaic virus ("TMV")) may be used; when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used; when generating cell lines that contain multiple copies of a the antigen coding sequence, SV40-, BPV- and EBV-based vectors may be used with an appropriate selectable marker.

Please insert the following replacement paragraph starting at page 13, line 6:

A variety of host-expression vector systems may be utilized to express a Ra12 fusion protein coding sequences. These include, but are not limited to, microorganisms such as bacteria (*e.g.*, *E. coli, B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing a coding sequence; yeast (*e.g.*, *Saccharomycdes, Pichia)* transformed with recombinant yeast expression vectors containing a coding sequence; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing a coding sequence; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing a coding sequence; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3 cells transformed with suitable expression vectors). The expression elements of these systems vary in their strength and specificities.

The specification at page 13, line 16 has been objected to for the phrase "mammalian cell system." Therefore, Applicants respectfully request withdrawal of this objection.

Please insert the following replacement paragraph starting at page 16, line 27:

B7

Thus, the terms such as "Ral2 polypeptide" or "Ral2 polypeptide sequence" as used herein refer to native Ral2 polynucleotide sequences (*e.g.*, SEQ ID NO:3), fragments thereof (*e.g.*, SEQ ID NO:17 or 18), or any variants thereof. Functionally, a Ral2 polypeptide has the ability to produce a fusion protein, and its ability to produce a fusion proteins in host cells may be enhanced or unchanged, relative to the native Ral2 polypeptide (*e.g.*, SEQ ID NO:4), or may be diminished by less than 50%, and preferably less than 20%, relative to the native Ral2 polypeptide.

Please insert the following replacement paragraph starting at page 20, line 7:

B8

One of skill would recognize that modifications can be made to the recombinant nucleic acids and fusion polypeptides without diminishing their biological activity. Some modifications may be made to facilitate the cloning, expression, or incorporation of the tag molecule into a fusion polypeptide. Such modifications are well known to those of skill in the art and include, for example, a methionine added at the amino terminus to provide an initiation site, or additional amino acids (*e.g.*, polyHistidine ("poly His")) placed on either terminus to create conveniently located restriction sites or termination codons or purification sequences.

IN THE CLAIMS:

Please cancel claims 7-9,12, 17-26 and 30 without prejudice to future prosecution.

Please amend claims 1, 10, 11, 13, and 27 as follows.

B9

1. (Once amended) A recombinant nucleic acid molecule that encodes a fusion polypeptide, the recombinant nucleic acid molecule comprising a Ral2